Evaluation of Cognitive Enhancing Activity of *Piper Longum* Fruit Extract on Albino Rats

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**ABSTRACT**
To investigate the cognitive enhancing activity of ethanolic fruit extract of *Piper longum* two methods are used. *Piper longum* were subjected to preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The dried fruits were subjected to successive solvent extraction with 70% of ethanol. The extract revealed the presence of tannins, lignans, and volatile oils. In the pharmacological screening the extract was used for the evaluation of cognitive enhancing activity using elevated plus maze and passive avoidance task methods using Donepezil as standard by using the parameters of step down and transfer latency. Induction was carried out by Diazepam for 7 days. Alcoholic extract showed significant effect when compared to control, their was significant increase in the step down latency and decrease in the transfer latency which was as effective as that of standard drug.

**Keywords:** *piper longum*, donepezil, diazepam, stepdown, transfer latency.

**INTRODUCTION**
Herbal Medicine, sometimes referred to as Herbalism or Botanical Medicine, is the use of herbs for their therapeutic or medicinal value. An herb is a plant or plant part valued for its medicinal, aromatic quality. The World Health Organization (WHO) estimates that 4 billion people, 80% of the world population, presently use herbal medicine for some aspect of primary health care. Herbal medicine is a major component in all indigenous peoples' traditional medicine and a common element in Ayurveda, homeopathic, naturopathic, traditional oriental, and Native American Indian medicine. Pippali consists of the dried, immature, catkin-like fruits with bracts of *Piper longum* Linn. (Fam. Piperaceae), a slender, aromatic climber with perennial woody roots, occurring in hotter parts of India from central Himalayas to Assam up to lower hills of West Bengal and ever green forests of Western ghats as wild, and also cultivated in North East and many parts of the South. Fruit greenish-black to black, cylindrical, 2.5 to 5 cm long and 0.4 to 1 cm thick, consisting of minute sessile fruits, arranged around an axis; surface rough and composite; broken surface shows a central axis and 6 to 12 fruitlets arranged around an axis; taste, pungent producing numbness on the tongue; odour, aromatic.

**Chemical Constituents and Components**
Main chemical components are piperine, rutin, beta-caryophyllene, piperyline, pipereoline, piperamine, sabinene, chavicin, pinene, phellandrene, pentadecane, beta-bisabolene, linalool and limonene.

**MATERIALS AND METHODS**
Collection of plant material
The dried fruits of *piper longum* were purchased from Munniyapa Aruvedha Krishnagiri. The plant was authenticated by Prof. P. Jayaraman, Ph.D. Director, National Institute Of Herbal Sciences Chennai.

**EXTRACTION OF CRUDE DRUGS**
The powdered fruit material was extracted with methanol using soxhlet apparatus and the extract obtained was then concentrated on the water bath. In successive solvent extraction, dried material was extracted with methanol. For extraction, 150gm of powdered stem was packed in thimble containing whatmann filter paper and extracted with methanol (60°C-70°C) in soxhlet apparatus for the period till all the crude substances were extracted. The extract thus obtained was concentrated with the help of rotatary vacuum evaporator.
Selection of Animals for testing the cognitive enhancing activity
Animal care and handling as per CPCSEA guideline
Male Wistar albino rats of weight 150-250 grams were selected, procured from Padmavathi college animal house. The animals were acclimatized to the standard laboratory conditions in well cross ventilated animal house at temperature 25±2°C relative humidity 44–56% and light and dark cycles of 10 and 14 hours respectively for 1 week before and during the experiments. The animals were fed with standard diet and water adlibitum. The experiments were approved by CPCSEA and the institutional ethics committee. Food was withdrawn 18hours before the start of the activity.

Method for Testing Cognitive Enhancing Activity
The animals were divided into Five groups of six in each group
- **Group I (Normal control)**: Normal given water daily for 7 days.
- **Group II (Positive control)**: given extract for sub group 2 and standard drug for sub group 3.
- **Group III (Negative control)**: Induction with Diazepam.
- **Group IV**: treated group in which sub group 5 was treated with extract and sub group 6 with standard drug.

### Table 1: Grouping of Animals

<table>
<thead>
<tr>
<th>Main Groups</th>
<th>Sub-groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>Normal control 1. No induction and no Treatment</td>
</tr>
<tr>
<td>Group II</td>
<td>Positive control 2. Only Piper Longum 3. Only Standard</td>
</tr>
<tr>
<td>Group III</td>
<td>Negative control 4. Induction with Diazepam</td>
</tr>
<tr>
<td>Group IV</td>
<td>Treated group: Induced with Diazepam Treatment with extracts 5. Piper Longum Fruit Extract 6. Standard</td>
</tr>
</tbody>
</table>

Procedure
The animals were trained on the 0(zero) day and the acquisition of memory was tested on day1, later all the animals were dosed with respective drugs and kept in their home cage. Dosing is continued for 7days and on 7th day animals were subjected to retention test 25min.after the last dose for evaluating passive avoidance task(step down latency) and elevated plus maze(transfer latency).

Screening test for memory
**Model 1**
**passive avoidance task (step down latency)**
Pole climbing apparatus chamber is used for passive avoidance response where pole is replaced by a wooden platform fixed on electrified grid floor. when rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4-5 trials the animals acquired the passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 sec. SDL is defined as time in sec taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor.

**Model 2**
**Elevated plus maze (transfer latency)**
An elevated plus maze consists of two open arms and two closed arms with an open roof. The maze was elevated to a height of 50cm. the animals were individually placed at the end of either of the open arms and the time taken for the animal to move from open to closed arm (transfer latency) was noted on zero day. The animals were allowed to explore the apparatus for 30sec. after 24h of first exposure TL was noted on day1 of the study for determining the acquisition. The criterion was reached when the animal moved in to the closed arms in a very short period keeping the cut off time of 60sec.

Statistical analysis
The SDL and TL were analysed using the students paired "t" test, twotailed. later the inflexion ration was calculated for the TL which was analysed using one way analysis of variance(ANOVA),Followed by Dunnets "t" test for individual comparision of groups with diazepam induced and controlled groups.
RESULTS

Table 1: Mean Values of Transfer latency of various drug treated groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>S.NO. OF SUB GROUP</th>
<th>SUB GROUP</th>
<th>TRANSFER LATENCY (in Sec)</th>
<th>Before day 1</th>
<th>after day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>01</td>
<td>Normal control</td>
<td>22.36±1.634</td>
<td>6.492±0.5862</td>
<td></td>
</tr>
<tr>
<td>Positive control</td>
<td>02</td>
<td>Extract</td>
<td>52.82±2.263</td>
<td>19.04±4.447</td>
<td></td>
</tr>
<tr>
<td>Negative control</td>
<td>03</td>
<td>Standard</td>
<td>28.89±6.007</td>
<td>14.90±1.169</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>04</td>
<td>Diazepam</td>
<td>24.71±4.325</td>
<td>47.12±5.485</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>05</td>
<td>Extract</td>
<td>37.43±1.665</td>
<td>15.48±7.693</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>06</td>
<td>standard</td>
<td>21.30±9.572</td>
<td>14.90±1.169</td>
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</tr>
</tbody>
</table>

Table 2: Mean Values of step down latency of various drug treated groups

<table>
<thead>
<tr>
<th>GROUP</th>
<th>S.NO. OF SUB GROUP</th>
<th>SUB GROUP</th>
<th>STEP DOWN LATENCY (in Sec)</th>
<th>Before day 1</th>
<th>after day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>01</td>
<td>Normal control</td>
<td>17.41±3.014</td>
<td>25.57±2.700</td>
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</tr>
<tr>
<td>Positive control</td>
<td>02</td>
<td>Extract</td>
<td>14.89±1.442</td>
<td>27.82±0.6974</td>
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<tr>
<td>Negative control</td>
<td>03</td>
<td>Standard</td>
<td>16.76±1.799</td>
<td>60.00±0.0</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>04</td>
<td>Diazepam</td>
<td>27.43±3.445</td>
<td>11.73±2.402</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>05</td>
<td>Extract</td>
<td>17.46±3.162</td>
<td>41.19±1.782</td>
<td></td>
</tr>
<tr>
<td>Treated group</td>
<td>06</td>
<td>standard</td>
<td>32.04±2.235</td>
<td>60.00±0.0</td>
<td></td>
</tr>
</tbody>
</table>

Mean Graph of Transfer Latency

Mean Graph of Step down Latency

DISCUSSION
AD is a progressive and fatal neurodegenerative disorder manifested by cognitive and memory deterioration, progressive impairment of routine activities of living, and a variety of routine activities of living, and a variety of neuropsychiatric symptoms and behavioural disturbances. The clinical features of AD are an amnesic type of memory impairment, deterioration of language and visuo spatial deficits. Motor and sensory abnormalities, gait disturbance and seizures are uncommon until the last phase of the disease. Despite the severity and high prevalence of this disease ,allopathic system of medicine is yet to provide a satisfactory antidote. Therefore, we were motivated to explore the new approach in the Indian traditional system to manage this deadly disease (AD).

Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory impairment in patients with the senile dementia are due to selective and irreversible deficiency in the cholinergic functions in the brain. This serves as a rationale for the use of AChE inhibitors for the symptomatic treatment of AD in its early stages. There are extensive evidences linking decreased brain cholinesterase activity and improvement in memory. cognitive dysfunction has been shown to be associated with impaired cholinergic function and the facilitation of central cholinergic activity with improved memory. Selective loss of cholinergic neurons and decrease in cholineacetyltransferase activity was reported to be a characteristic feature of senile dementia of Alzheimer’s type. It has been observed that elderly patients suffering from Alzheimer’s disease showed reduction in symptoms up on chronic use of anti-inflammatory drugs. Epidemiological studies have already confirmed that non-steroidal anti-inflammatory reduced the incidence of AD. *piper longum* have been proved as anti-inflammatory agents ,which might protect from the development of inflammatory lesions in brain.

In the present study, the fruits of *piper longum* were subjected to phytochemical investigation and cognitive enhancing activity. The ethanolic extract when tested for preliminary phytochemical investigation showed presence of alkaloids and amides, volatile oils , lignans, esters.

Cognitive Enhancement Activity was done by step down and transfer latency method, impairment of memory consolidation was done by diazepam. Chronic exposure to diazepam for 7 days produced a significant decrease in step down latency and increased the time of latency in elevated plusmaze. The alcoholic extract(3mg/kg.B.W) showed significant effect when compared to control, there was significant increase in step down latency and decrease in the transfers latency but was not so effective as that of standard drug. This suggested that application of diazepam disrupts the acquisition, retention and consolidation of learning task which was reversed by alcoholic extracts and standard. The result obtained has shown significant cognitive enhancing activity of alcoholic extract drug. Daily administration of extracts significantly attenuated the amnesic effect of diazepam.

**CONCLUSION**

In the present study fruits of *piper longum* were subjected to preliminary phytochemical investigation and pharmacological screening of cognitive enhancing activity. The dried fruits were subjected to successive solvent extraction with 70% of ethanol. The extract revealed the presence of tannins, lignans , and volatile oils. In the pharmacological screening the extract was used for the evaluation of cognitive enhancing activity using elevated plus maze and passive avoidance task methods using Donepezil as standard by using the parameters of step down and transfer latency. Induction was carried out by Diazepam for 7 days . Alcoholic extract showed significant effect when compared to control, their was significant increase in the step down latency and decrease in the transfer latency which was as effective as that of standard drug. Hence it can be concluded that fruits of *piper longum* possess cognitive enhancing activity.

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